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A monoclonal antibody against an activation epitope on mouse integrin chain β_1 blocks adhesion of lymphocytes to the endothelial integrin $\alpha_6\beta_1$

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ABSTRACT **We have generated a monoclonal antibody (mAb), 9EG7, against mouse endothelial cells that blocks adhesion of lymphocytes to endothelial cells. Sequencing of four tryptic peptides of the purified antigen revealed its identity with the integrin chain β_1. The only β_1 integrin that is known to mediate cell–cell adhesion is $\alpha_4\beta_1$ (VLA-4). This is not the integrin that is functionally defined by the mAb 9EG7 on endothelial cells. First, α_4 is not present on the analyzed endothelial cells. Second, mAb 9EG7 does not block the cell-adhesion function of $\alpha_4\beta_1$ on the nonactivated mouse lymphoma L1-2. Thus, the mAb 9EG7 can functionally distinguish between different β_1 integrins and defines a β_1 integrin other than $\alpha_4\beta_1$ as a newly discovered cell–cell adhesion molecule. This integrin is most likely $\alpha_6\beta_1$, since an antibody against the α_6 chain blocks lymphocyte adhesion to the same degree as the mAb 9EG7, the effect of both antibodies is not additive, and the α_6 chain is coprecipitated with β_1 in 9EG7 immunoprecipitations. Surprisingly, activation of $\alpha_4\beta_1$ on L1-2 cells with phorbol ester or Mn^{2+} allows blocking of $\alpha_4\beta_1$-mediated adhesion of L1-2 cells to endothelial cells with mAb 9EG7. Furthermore, only the activated $\alpha_4\beta_1$ heterodimer, but not the unactivated complex, is detectable with 9EG7 in immunoprecipitations and by flow cytometry. Thus, mAb 9EG7 defines an epitope on integrin chain β_1, which is accessible on the $\alpha_4\beta_1$ heterodimer only after activation of this integrin.**

Lymphocytes leave the bloodstream to enter into sites of inflammation (1) or to fulfill their survey function by migrating into various lymphoid and nonlymphoid organs—a process called lymphocyte homing (2). The central step that initiates these migration processes is adhesion of lymphocytes to endothelial cells. Numerous cell adhesion molecules (CAMs) are involved in this process and probably function in a cascade of molecular interactions (3, 4). These CAMs belong to several protein families of adhesion molecules.

The selectins form a family of three carbohydrate binding CAMs, which mediate early events in the binding of leukocytes to endothelial cells (5, 6). A very large family of CAMs belongs to the immunoglobulin supergene family (7). Four of its members are expressed on endothelial cells: the cytokine-inducible ICAM-1 (8) and VCAM-1 (9) and the constitutively expressed ICAM-2 (10) and PECAM-1 (11).

The integrins are $\alpha\beta$ heterodimers for which 8 β chains and 14 α chains have been identified (12). The majority of the known integrins function as receptors for extracellular matrix proteins; only six have been described to directly support cell–cell adhesion. These are the β_2 integrins LFA-1 ($\alpha_L\beta_2$), Mac-1 ($\alpha_M\beta_2$), and p150/95 ($\alpha_X\beta_2$) (13), which are specific for leukocytes; the platelet integrin $\alpha_{IIb}\beta_3$, which mediates platelet aggregation via bridging of two such integrins with fibrinogen (14); and the two α_4 integrins $\alpha_4\beta_1$ (VLA-4) (15) and $\alpha_4\beta_p$ (LPAM-1) (16), which are expressed on lymphocytes. All these integrins, except the platelet integrin $\alpha_{IIb}\beta_3$, function as leukocyte adhesion molecules, which bind to ligands on endothelial cells. These ligands are the immunoglobulin superfamily members ICAM-1 (for LFA-1 and Mac-1), ICAM-2 (only for LFA-1), and VCAM-1 (for $\alpha_4\beta_1$ and with lower affinity for $\alpha_4\beta_p$) (17, 18).

This large variety of CAMs on leukocytes and endothelial cells is necessary to regulate the complex process of leukocyte extravasation. Different CAMs preferentially mediate the binding of different categories of leukocytes to endothelial cells. Furthermore, leukocyte adhesion to endothelium probably is a multistep process requiring multiple receptor–ligand pairs that act sequentially (3, 4). For example, the selectins mediate a very early step in leukocyte adhesion to the vessel wall, while LFA-1/ICAM-1 can stabilize adhesion in a subsequent step (19, 20).

We have searched for further endothelial CAMs that mediate adhesion of lymphocytes. We have generated the monoclonal antibody (mAb) 9EG7, which defines a β_1 integrin on endothelial cells as a receptor for mature lymphocytes. This integrin is $\alpha_6\beta_1$. Furthermore, the 9EG7 antibody binds to and blocks the function of another cell adhesion-mediating β_1 integrin, $\alpha_4\beta_1$, only if this integrin is in an activated state. Thus, 9EG7 defines an activation epitope on the integrin chain β_1.

MATERIALS AND METHODS

Cells. The following cell lines were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum (FCS): mouse endotheliomas bEnd.3 and sEnd.1 (21), all hybridomas, the human lymphoma Ramos (22), and the mouse endothelial cell line TME (23). The mouse lymphoma L1-2 (16) was cultured as described (16).

Antibodies. The following mAbs against mouse integrin chains were used: PS/2 (24) (anti-α_4), R1-2 (16) (anti-α_4), GoH3 (25) (anti-α_6), and EA-1 (26, 27) (anti-α_6). Other mAbs were K20 (28) (anti-human integrin chain β_1) and MK-2 (29) (anti-mouse VCAM-1). Rabbit antibodies against a peptide corresponding to the C-terminal domain of the chicken integrin chain β_1 were obtained from Richard O. Hynes (MIT). The mAb 9EG7 (IgG2a) was produced by immunizing rats with TME cells in phosphate-buffered saline (PBS), using Alu-Gel-S (Serva) as adjuvant. Hybridoma production was done essentially as described (30) except that the mouse myeloma Sp2/0 was used for the fusion. Hybridoma supernatants were screened in cell-surface ELISA assays on TME

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Abbreviations: CAM, cell adhesion molecule; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate.

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cells and sEnd.1 cells by indirect immunoperoxidase staining as described (31). Monovalent Fab fragments of mAbs were generated as described (32).

Cell Adhesion Assay. Adhesion of freshly isolated lymphocytes from mouse mesenteric lymph nodes to the monolayer of TME cells was assayed in 96-well microtiter plates essentially as described (23). Quantification was done either with lymphocytes that had been labeled with the DNA-staining fluorescence dye H33342 (Hoechst) in a fluorescence ELISA reader or with unlabeled lymphocytes by counting the bound cells under a microscope. Antibody incubations with TME cells before the assay were done for 45 min at 37°C.

Purification and Sequencing of the 9EG7 Antigen. TME cells (3×10^9 cells) were nonenzymatically harvested, washed once in PBS, and lysed for 20 min at 4°C in 240 ml of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS buffer) (3% CHAPS in 20 mM Tris·HCl, pH 8.0/150 mM NaCl/1 mM $CaCl_2$/1 mM phenylmethylsulfonyl fluoride). The extract was centrifuged for 15 min at $10,000 \times g$ at 4°C and incubated for 3 h at 4°C with 800 μl of CNBr Sepharose beads (Pharmacia) conjugated with 2.4 mg of mAb 9EG7. Beads were washed five times with CHAPS buffer and eluted with 1 ml of SDS buffer [1% SDS in 100 mM NH_4HCO_3 (pH 7.4)] for 30 min at 55°C. Eluted material was lyophilized, dissolved in 200 μl of H_2O, precipitated with 90% ethanol, lyophilized again, and treated with trypsin. Resulting peptides were separated by reversed-phase HPLC and sequenced (Applied Biosystems 477A protein sequencer).

Immunoprecipitations and immunoblots were done essentially as described (33).

Flow Cytometry. Antibody incubations and washing steps were performed at 4°C in 20 mM Hepes, pH 7.4/150 mM NaCl/3% dialyzed FCS/0.1% azide, containing either 1 mM each Mg^{2+} and Ca^{2+} or 0.2 mM Mn^{2+}. Phorbol 12-myristate 13-acetate (PMA) activation was done with 1 ng of PMA per ml for 15 min at 37°C immediately before the assay. Cells were analyzed in a FACScan flow cytometer (Becton Dickinson).

RESULTS

A mAb Against Endothelial Cells Blocks Lymphocyte Adhesion. Freshly isolated lymphocytes from mouse mesenteric lymph nodes bind specifically to the lymph node-derived endothelial cell line TME (23). In contrast, lymphocyte binding to the mouse endothelioma sEnd.1 cells is very weak. To identify CAMs that mediate the binding of lymphocytes on TME cells, we raised mAbs against TME cells by immunizing rats with intact cells and then screening for antibodies that detect cell-surface antigens on TME but not, or only weakly, on sEnd.1 cells.

Seventy mAbs were found that recognized antigens on the surface of intact TME cells as analyzed in indirect immunoperoxidase staining experiments. These antibodies stained sEnd.1 cells weakly or not at all. Only one of them, designated 9EG7, was found to partially inhibit (up to 25%) lymphocyte binding to TME cells when plain hybridoma supernatant was incubated with TME cells before the cell adhesion assay (Fig. 1). None of the other 69 hybridoma supernatants had any effect in this assay. To demonstrate that the inhibitory activity in the 9EG7 supernatant was due to the mAb, the supernatant was depleted of the antibody by incubation with rabbit anti-rat IgG, which was bound to protein A-Sepharose. This procedure removed the 9EG7 antibody completely, as was tested by indirect immunoperoxidase staining of TME cells (data not shown). The depleted supernatant had lost its cell binding inhibitory activity (Fig. 1). Monovalent Fab fragments of the 9EG7 antibody at a concentration of 25 μg/ml inhibited lymphocyte binding to the same extent as 9EG7 hybridoma supernatant (Fig. 1), excluding an indirect effect of the antibody by crosslinking the antigen.

Maximal inhibition (25%) of lymphocyte binding was found with 9EG7 antibody at 160 μg/ml (data not shown). An additional adhesion mechanism on TME cells could be demonstrated with the anti-mouse VCAM-1 antibody MK-2 (29). This antibody inhibited lymphocyte binding to 38% (Fig. 1). The inhibitory effect of both antibodies was additive, causing 63% inhibition, when TME cells were incubated with both antibodies prior to cell binding (Fig. 1).

Identification of the Antigen as the Integrin Chain β_1. In immunoprecipitations from cell lysates of TME cells metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine, 9EG7 recognizes a single protein of apparent molecular mass 120 kDa (Fig. 2*A*). This antigen is also recognized in immunoblots; however, reduction of the antigen destroys the 9EG7 epitope (data not shown), indicating that the antibody recognizes a conformation-specific epitope.

To identify the antigen, we decided to partially sequence the protein. To this end, the antigen was purified from TME cell lysates by using the 9EG7 antibody conjugated to CNBr Sepharose as an immunoaffinity matrix. From 3×10^9 cells, 20 μg of the protein was obtained as determined by PAGE and Coomassie staining. This material was digested with

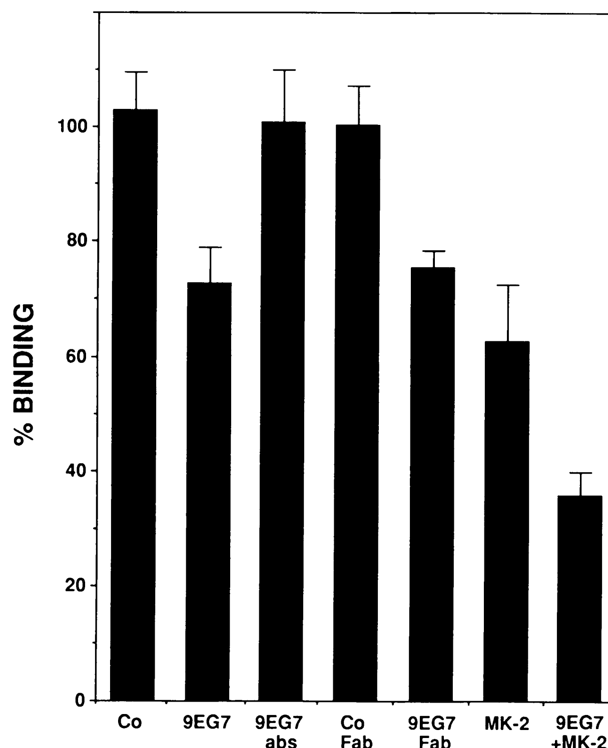

FIG. 1. 9EG7 inhibits lymphocyte adhesion to TME cells. Cell adhesion assays were performed with freshly isolated lymphocytes from mouse mesenteric lymph nodes and the endothelial cell line TME as described. Adhesion was quantitated by counting adherent lymphocytes under the microscope; each measurement was done in triplicate and one of three similar series of experiments is shown in each graph. Binding in the absence of any added antibodies was defined as 100% and corresponded to 2280 (columns 1–3), 2320 (columns 4 and 5), and 3900 (columns 6 and 7) bound lymphocytes per mm^2. TME cells were incubated for 45 min before incubation with lymphocytes with an isotype-matched control hybridoma supernatant (Co), 9EG7 hybridoma supernatant (9EG7), 9EG7 hybridoma supernatant from which the mAb had been removed by preincubation with rabbit IgG anti-rat IgG conjugated to protein A-Sepharose (9EG7 abs), monovalent Fab fragments (25 μg/ml) of an isotype-matched control antibody (Co Fab), 9EG7 Fab fragments (25 μg/ml) (9EG7 Fab), 1:100 diluted ascites of the anti-VCAM-1 mAb MK-2 (MK-2), and a mixture of both antibodies (9EG7+MK-2).

trypsin, the resulting peptides were separated by HPLC, and four different peptides were sequenced. Each of the four sequences was identical to part of the sequence of the mouse integrin chain β_1. These were located at amino acid positions 185–200 (XPCTSEQXCTSPFSYK), 605–622 (FQXPT-CETCQTCLGVCAE), 755–764 (WDTGENPIYK), and 657–672 (EKLPQPVQVDPVTHCK), with the N-terminal amino acid of the mature protein taken as position 1.

The identity of the 9EG7 antigen with the integrin chain β_1 was further tested in immunoprecipitations with a rabbit antiserum against a peptide coding for C-terminal amino acids of the cytoplasmic tail of chicken β_1. Fig. 2*B* shows that the 9EG7 antigen and mouse β_1 are identical in their electrophoretic mobility. Furthermore, three rounds of preincubation of cell extracts with the 9EG7 antibody removed 90% of the 9EG7 antigen as well as 90% of the β_1 chain (Fig. 2*B*), while preincubation with a control antibody did not remove these proteins. We conclude that the 9EG7 antigen is identical to the mouse integrin chain β_1.

The Corresponding α Chain Is α_6. The only β_1 integrin that has been reported to directly mediate cell–cell adhesion is $\alpha_4\beta_1$ (VLA-4) on lymphocytes, which binds to VCAM-1 on endothelial cells. VLA-4 has not been found on endothelial cells. In agreement with this, we could not detect α_4 by indirect immunoperoxidase staining on the surface of TME cells by using the anti-α_4 mAbs PS/2 and R1-2 (data not shown).

Recently, an endothelial CAM, defined by the mAb EA-1, which blocks the binding of progenitor T cells to mouse endothelial cells, was identified as the integrin chain α_6 (27). Using the EA-1 antibody, we could clearly detect α_6 on the surface of TME cells by indirect immunoperoxidase staining on intact cells (data not shown). We therefore tested whether EA-1 could also inhibit the binding of lymphocytes from mesenteric lymph nodes to TME cells. Indeed, EA-1 and 9EG7 blocked cell adhesion to the same degree of 32% (±8%) and 32% (±5%), respectively. The inhibitory activity of both antibodies was not additive (36% ± 3%). This suggests that it is the integrin $\alpha_6\beta_1$ that mediates the binding of lymph node lymphocytes to TME cells.

In agreement with this, we could directly demonstrate that mAb 9EG7 binds to the $\alpha_6\beta_1$ heterodimer on TME cells. When β_1 integrins immunoprecipitated with 9EG7 from TME cells were analyzed in immunoblots, the α_6 chain was detected by the mAb GoH3 (data not shown).

The Anti-β_1 mAb 9EG7 Recognizes an Activation Epitope on $\alpha_4\beta_1$ (VLA-4). We tested whether 9EG7 could also block the cell adhesion function of the integrin $\alpha_4\beta_1$ on lymphocytes. For these tests, we used the lymphoma cell line L1-2, which expresses $\alpha_4\beta_1$ but not $\alpha_4\beta_p$ (34). As shown in Fig. 3, binding of L1-2 cells to tumor necrosis factor-activated bEnd.3 cells was strongly inhibited when the lymphoma cells were preincubated with the anti-α_4 mAb PS/2. However, no inhibition was seen when the L1-2 cells were preincubated with the mAb 9EG7 (Fig. 3). Thus, the 9EG7 epitope on the β_1 chain of the integrin $\alpha_6\beta_1$ is (*i*) not present on $\alpha_4\beta_1$, (*ii*) not accessible on $\alpha_4\beta_1$, or (*iii*) accessible but not relevant for the function of $\alpha_4\beta_1$.

To distinguish between these possibilities, we tested whether 9EG7 could block the function of activated $\alpha_4\beta_1$. It has been proposed that phorbol esters and also Mn^{2+}, which both activate the adhesiveness of several integrins, act via alterations of the conformation of integrins. Therefore, L1-2 cells were treated either with PMA (1 ng/ml) for 15 min prior to antibody incubation or with 0.2 mM Mn^{2+} during antibody incubation. Both treatments enhanced the binding of L1-2 cells to tumor necrosis factor-activated bEnd.3 cells (Fig. 3). This enhanced binding was mediated by $\alpha_4\beta_1$, since it could be blocked by the anti-α_4 mAb PS/2. In contrast to the cell binding via unactivated $\alpha_4\beta_1$, enhanced binding via activated

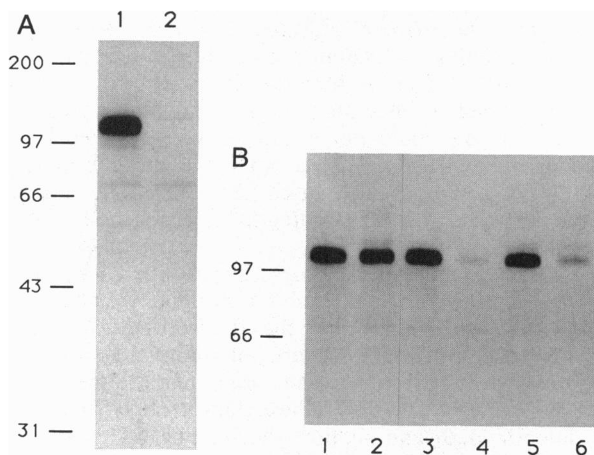

FIG. 2. Detection of the 9EG7 antigen by immunoprecipitation. (*A*) TME cells were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine and detergent extracts were immunoprecipitated with 9EG7 (lane 1) or an isotype-matched control antibody (lane 2). (*B*) Cell lysates of TME cells metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine were immunoprecipitated with mAb 9EG7 (lane 1) and a polyclonal rabbit antiserum against the cytoplasmic tail of the chicken integrin chain β_1 (lane 2). In immunodepletion experiments, the cell extract was preincubated three times with a control mAb (lanes 3 and 5) or with the 9EG7 mAb (lanes 4 and 6), and after removal of antibody–antigen complexes the depleted extracts were split into halves and each half was immunoprecipitated either with 9EG7 (lanes 3 and 4) or with the polyclonal anti-β_1 antiserum (lanes 5 and 6). Immunoprecipitates were electrophoresed on 8% polyacrylamide gels under reducing conditions and analyzed by fluorography; relative molecular masses (in kDa) are marked on the left.

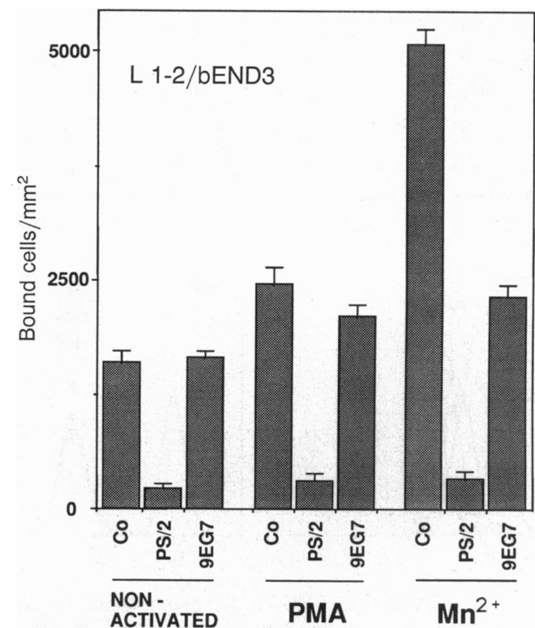

FIG. 3. The 9EG7 antibody blocks cell adhesion mediated by $\alpha_4\beta_1$ only if $\alpha_4\beta_1$ is activated. Cell adhesion assays were performed with the mouse lymphoma L1-2 and tumor necrosis factor-activated bEnd.3 cells as described. L1-2 cells were either directly used (nonactivated) or treated with PMA (1 ng/ml) for 15 min prior to antibody incubation (PMA) or the antibody incubation and all subsequent steps of the adhesion assay were performed in the presence of 0.2 mM Mn^{2+} (Mn^{2+}). Before the assay L1-2 cells were incubated for 30 min with an isotype-matched control antibody in culture medium (150 μg/ml) (Co), the anti-α_4 mAb PS/2 (60 μg/ml), or 9EG7 in culture medium (160 μg/ml).

$\alpha_4\beta_1$ could be partially blocked by the mAb 9EG7 (Fig. 3), arguing for accessibility of the 9EG7 epitope only in the activated conformation of $\alpha_4\beta_1$.

This could be confirmed by flow cytometry. Treatment of L1-2 cells with PMA or Mn^{2+} in a manner similar to that described above increased the binding of 9EG7 to L1-2 cells but did not influence the binding of mAb PS/2 (Fig. 4). Since 9EG7 also recognizes human β_1, we could perform the same analysis for the human lymphoma Ramos. A result similar to that for L1-2 cells was observed; again Mn^{2+} treatment of cells enhanced the binding of 9EG7 more strongly than treatment with PMA (Fig. 4). No effect was seen on the binding of the mAb K20 against human β_1 chain (Fig. 4).

The fact that the 9EG7 epitope is indeed present on the β_1 chain of the integrin $\alpha_4\beta_1$ was further demonstrated by immunoblotting. The $\alpha_4\beta_1$ heterodimer was isolated from L1-2 cells by immunoaffinity chromatography with the anti-α_4 mAb PS/2 immobilized to CNBr Sepharose and analyzed in immunoblots with mAb 9EG7. The β_1 chain coprecipitated with the α_4 chain was clearly detected by the mAb 9EG7 (data not shown). The effect of Mn^{2+} on the accessibility of the 9EG7 epitope in the $\alpha_4\beta_1$ complex was directly demonstrated in immunoprecipitations. The mAb 9EG7 coprecipitated α_4 together with the β_1 chain in the presence of Mn^{2+} but not in its absence (Fig. 5).

Binding of 9EG7 as well as of lymphocytes to TME cells (which do not express α_4 chains) could also be slightly enhanced by Mn^{2+}, although not by PMA. This enhanced lymphocyte binding was blocked by mAb 9EG7 (data not shown).

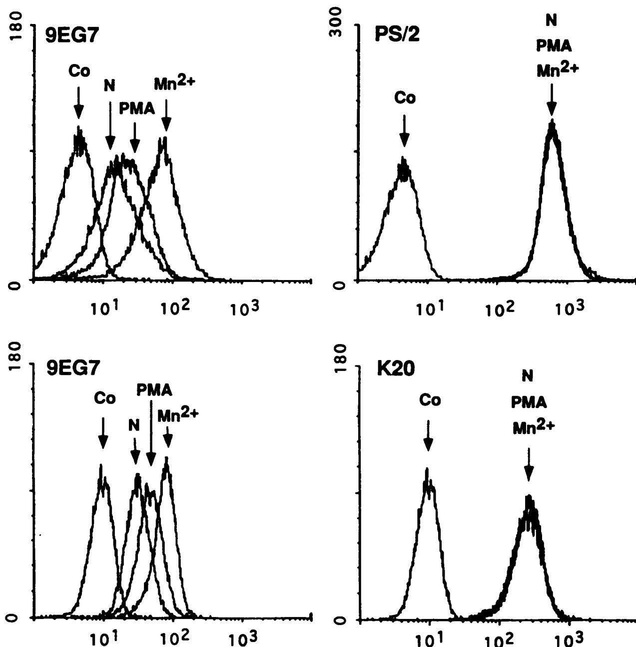

FIG. 4. Binding of mAb 9EG7 to lymphoma cells is induced by PMA and Mn^{2+}. Flow cytometry was performed on the murine lymphoma L1-2 (*Upper*) and the human lymphoma Ramos (*Lower*) using the mAbs 9EG7, PS/2 (anti-mouse α_4 chain), and K20 (anti-human β_1 chain) as indicated. mAbs 9EG7 and PS/2 were directly fluorescein isothiocyanate (FITC) labeled and mAb K20 was detected by a second FITC-labeled goat anti-mouse antibody. Nonspecific binding was controlled either with an isotype-matched FITC-conjugated control antibody or with FITC-conjugated goat anti-mouse IgG (Co). Antibody incubations were done with untreated cells in the presence of 1 mM Mg^{2+} and 1 mM Ca^{2+} (N), PMA-activated cells in the same buffer (PMA), or untreated cells in the presence of 0.2 mM Mn^{2+} and in the absence of Mg^{2+} and Ca^{2+} (Mn^{2+}).

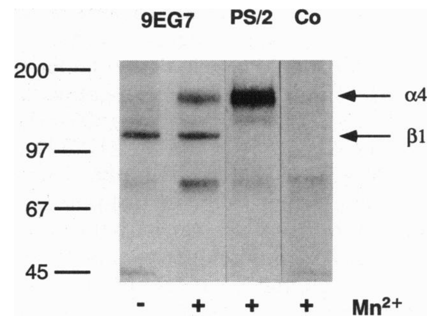

FIG. 5. The 9EG7 epitope is present in the $\alpha_4\beta_1$ heterodimer but is only accessible after activation of this integrin. Cell lysates of L1-2 cells metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine were immunoprecipitated in the absence (lane −) and in the presence (lanes +) of 0.2 mM Mn^{2+} with mAb 9EG7, the anti-α_4 mAb PS/2, and an isotype-matched control antibody (Co) as indicated. Precipitated proteins were electrophoresed under nonreducing conditions and analyzed by fluorography. Positions of the α_4 and the β_1 chain are indicated. Relative molecular masses (in kDa) are indicated on the left.

DISCUSSION

We have generated a mAb against mouse endothelial cells that blocks lymphocyte adhesion to endothelial cells and that recognizes the integrin chain β_1. This defines a member of the β_1 integrins as an additional cell adhesion receptor for lymphocytes on endothelial cells.

All known β_1 integrins function as receptors for proteins of the extracellular matrix. The only β_1 integrin that has been shown so far to directly mediate cell–cell adhesion is the integrin $\alpha_4\beta_1$ (VLA-4) on lymphocytes and monocytes. These cells bind via $\alpha_4\beta_1$ to the cytokine-inducible VCAM-1 on endothelial cells. In addition to this role in the extravasation of leukocytes at sites of inflammation, the ligand pair $\alpha_4\beta_1$/VCAM-1 is involved in lymphocyte differentiation in long-term bone marrow cultures (24) and also functions outside the leukocyte system, mediating secondary myotube formation (35). The β_1 integrin that we have found on endothelial cells to mediate adhesion of lymphocytes is not $\alpha_4\beta_1$. First, we could not detect α_4 on endothelial cells; second, the mAb 9EG7 does not block the cell adhesion function of $\alpha_4\beta_1$ on nonactivated L1-2 cells. Thus, the mAb 9EG7 defines a second β_1 integrin as a cell–cell adhesion molecule.

This integrin is most likely $\alpha_6\beta_1$. The mAb EA-1 against the mouse integrin chain α_6 (27) inhibits lymphocyte adhesion to TME cells to the same degree that the mAb 9EG7 does, and the effects of both antibodies are not additive. Furthermore, we could demonstrate that the $\alpha_6\beta_1$ heterodimer can be affinity isolated with 9EG7. These data strongly suggest that the integrin $\alpha_6\beta_1$ can support the binding of lymph node-derived, mature lymphocytes to endothelial cells.

Our data extend the data of Imhof *et al.* (26), who demonstrated that the mAb EA-1 inhibits binding of progenitor T cells to the mouse endothelioma cell line eEnd.2. However, eEnd.2 cells express $\alpha_6\beta_1$ and $\alpha_6\beta_4$ (27) and it has not yet been determined which of the two mediates the binding of progenitor T cells.

The role $\alpha_6\beta_1$ plays on endothelial cells during lymphocyte extravasation *in vivo* remains to be defined. According to a current model, leukocytes adhere to endothelial cells in a multistep process, which is mediated by multiple receptor–ligand pairs (3, 4). The selectins are probably involved in very early interactions and may even initiate the whole process, while the leukocyte integrins, such as LFA-1, probably act at a later stage (19, 20). Since $\alpha_6\beta_1$ is constitutively expressed on endothelial cells throughout the organism (36), it is likely

that $\alpha_6\beta_1$ also acts at a later stage after initial binding has occurred. Just as it is speculated for LFA-I/ICAM-1, the $\alpha_6\beta_1$ integrin and its lymphocytic ligand might also be involved in the migration of lymphocytes on the endothelial cell surface, allowing the lymphocyte to move through the endothelial cell layer.

We were surprised to find that the mAb 9EG7 blocks the β_1-mediated adhesion of endothelial cells to lymphocytes, yet it does not block the function of $\alpha_4\beta_1$ on the nonactivated lymphoma L1-2. Despite this lack of adhesion blocking activity, we found that the 9EG7 epitope was present on the β_1 chain of the purified $\alpha_4\beta_1$ complex after dissociation of the α_4 chain. Thus, the 9EG7 epitope was either not relevant for the function of $\alpha_4\beta_1$ or not accessible in the heterodimeric complex.

To distinguish between these two possibilities, we tested whether treatments known to increase the binding affinity of integrins and probably alter their conformation would influence the accessibility of the 9EG7 epitope on $\alpha_4\beta_1$. Phorbol esters are well documented to increase the avidity of many integrins (12). For a number of integrins, Mn^{2+} has also been shown to enhance their ligand adhesiveness—as for the β_1 integrins VLA-6 (37), VLA-5 (38), and VLA-3 (39); the β_3 integrins II_bIII_a (40); vitronectin receptor (41); and the β_2 integrin LFA-1 (42). In the case of LFA-1, this effect of Mn^{2+} could be correlated with an altered conformation of the α chain since the epitope of mAb 24 on the α chain was inducible by Mn^{2+} (42). We have found a similar situation for the integrin $\alpha_4\beta_1$ and the mAb 9EG7, although in this case the mAb is directed against a β chain. Binding of 9EG7 to the mouse lymphoma L1-2 and the human lymphoma Ramos was enhanced either by activation of these cells with PMA or by addition of Mn^{2+}. Both treatments activated the adhesiveness of $\alpha_4\beta_1$, and this enhanced adhesiveness could be inhibited by 9EG7. The induction of the 9EG7 epitope on the $\alpha_4\beta_1$ complex could also directly be demonstrated in immunoprecipitations performed with 9EG7 in the absence or presence of Mn^{2+}.

Thus, the activation of integrin $\alpha_4\beta_1$ causes alterations of the β chain that can be detected by mAb 9EG7. Mapping of the epitope of the 9EG7 mAb may prove to be useful to understand how activation of β_1 integrins influences the structure of the β_1 chain.

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